Kidney International, Vol. 41 (1992), pp. 546–549

How does interleukin-1 activate cells?

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Interleukin-1 (IL-1) belongs to a family of cell activators termed cytokines. These are proteins which mediate the activation, differentiation or maturation of cells involved in immune and inflammatory responses [reviewed in 1]. The advent of cytokines has allowed elaborate networks to be established between a wide variety of cell types. This has greatly improved our understanding of the molecular and cellular events which occur during inflammation.

IL-1 can perhaps be considered the prototypical cytokine. It occurs in two forms, IL-1α and IL-1β, both with a molecular weight of 17000 daltons. The major cellular source of IL-1 is the activated mononuclear phagocyte and its many activities, both in vivo and in vitro indicate that it is a central mediator of inflammation [2, 3]. IL-1 is the most potent and multifunctional activator of cells so far described in cell biology. In vitro, IL-1 activates a large number of cell types. It will induce IL-2 production from lymphocytes. From connective tissue cells it induces prostaglandin and metalloproteinase production, as well as a range of other cytokines such as IL-6 and colony stimulating factors. A similar response is induced in mesangial and endothelial cells, where it also increases the expression of leukocyte adhesion molecules; in hepatocytes IL-1 increases the expression of several acute phase proteins. These effects provide an explanation for the many in vivo responses to IL-1, which include induction of fever, slow wave sleep, enhanced immune reactivity, neutrophilia and the acute phase response. Its effects in joints, where IL-1 induces resorption of cartilage and bone and leukocyte accumulation, have lead to IL-1 being implicated as a key mediator in rheumatoid arthritis. Despite these many activities being well documented, the molecular basis for the many potent and varied effects of IL-1 remains unclear. Elucidating the events which occur once IL-1 binds to cells has been a controversial area and there is still no consensus [4]. Determining these events remains a clear goal for our overall understanding of how IL-1 works.

IL-1 receptors

There are at least two distinct plasma membrane receptors for IL-1. The first to be described was the 80 kdal form, now termed type I [5]. This is the predominant form and occurs on T lymphocytes, connective tissue cells, endothelial cells and mesangial cells. It has been cloned and is a member of the immunoglobulin superfamily. Its structure did not yield any clues as to a possible post-receptor signalling mechanism. The second form, type II, was originally described on the pre-B cell line 70Z/3 [6], and has a molecular weight of 67 kdal. It is now known to occur mainly on bone-marrow derived cells. IL-1 receptors are present in low numbers compared to other hormone receptors (for example, T lymphocytes express only 1 to 2000 receptors per cell). In order to explain the dramatic changes in cellular activity which occur in response to IL-1, a major amplification in signal must occur following receptor binding.

G protein activation by IL-1

G proteins are guanine nucleotide binding proteins which couple receptors to effector systems such as adenylyl cyclase and phospholipases [7]. As many G proteins can be activated by a single occupied receptor, they provide a primary means of amplifying the response to a hormone. There is evidence for G protein activation in response to IL-1 [8–10]. In the murine thymoma line EL4.NOB-1, I have shown that IL-1 activates a G protein within one minute of binding its receptor [8]. Similarly, Chedid et al have provided evidence for a G protein in the signal for IL-1 in synovial fibroblasts, YT cells and 70Z/3 cells [9]. Much of the evidence is based on studies with pertussis toxin, a bacterial toxin which causes whooping cough. Pertussis toxin works by ADP-ribosylating the inhibitory G protein, Gi, which inactivates it. If the toxin inhibits a biological response to a hormone, Gi can then be implicated in the signal transduction pathway for that hormone. I have shown that pertussis toxin inhibits the induction of IL-2 from EL4 cells [8]. Chedid et al demonstrated that the toxin inhibited a range of IL-1 responses, including induction of prostaglandin release from fibroblasts and expression of IL-2 receptors on YT cells [9]. Dobson et al showed that pertussis toxin inhibited increases in diacylglycerol induced by IL-1 in EL4 cells [10]. Taken together, these reports suggest an implicit role for Gi in IL-1 signal transduction. Recently, however, I have found evidence which indicates that the inhibitory effect of pertussis toxin may be unrelated to effects on Gi.

The B oligomer of pertussis toxin inhibits IL-1 action

Pertussis toxin consists of six subunits [11]. The A subunit is an ADP-ribosyltransferase and mediates the inactivation of Gi. The remaining five subunits comprise the B oligomer of the toxin, which is important for toxin binding. Figure 1 shows how both the holotoxin and B oligomer potently inhibit the induction of IL-2 by IL-1 in EL4 cells. As the B oligomer is devoid of ADP-ribosylating activity its inhibitory effect cannot involve inactivation of a G protein. This implies that any modulatory effect of the holotoxin on IL-1 cannot be used as evidence to implicate a G protein in IL-1 action. The B oligomer was also found to inhibit IL-1-induced prostaglandin release from human

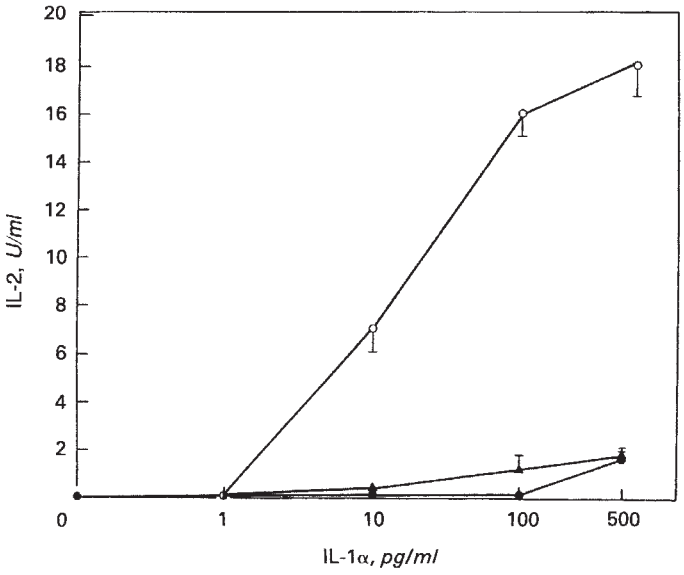

Fig. 1. *Effect of pertussis toxin or B oligomer on IL-1-induced IL-2 production by EL4 cells.* EL4 cells were preincubated for 4 hours at 37°C with holotoxin (●, 100 ng/ml), B oligomer (▲, 100 ng/ml) or no addition (○), washed and then incubated with increasing concentrations of IL-1 for 24 hours, after which supernatants were removed and assayed for IL-2 on CTLL cells as described previously [8]. Results are expressed as U/ml IL-2 and are representative of two separate experiments.

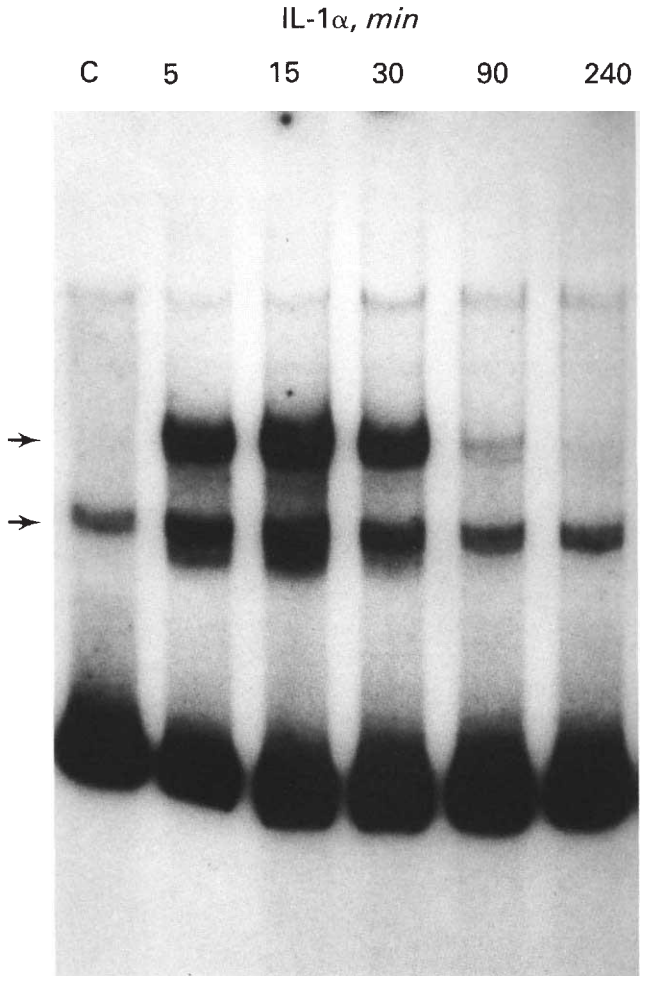

Fig. 2. *Time course of induction of NFκB by IL-1 in EL4 cells.* Cells (5×10^6 in 1 ml) were incubated times with IL-1α (10 ng/ml) for the indicated times, after which nuclear extracts were prepared as described [18]. NFκB binding activity was measured using a standard electrophoretic mobility assay technique [18]. In this method, 4 μg of nuclear extract protein was incubated with [^{32}P]-labelled DNA probes containing the NFκB motif for 30 minutes at room temperature. Protein-DNA complexes were then separated by electrophoresis and identified by autoradiography. The arrows indicate the retarded bands induced upon stimulation.

gingival fibroblasts (not shown), indicating that the effect of the toxin was not restricted to lymphocytes. Interestingly, neither toxin preparation affected phorbol ester-induced prostaglandin production (not shown) suggesting that the effect of B oligomer is somewhat specific for IL-1. The mechanism of action of the B oligomer is unclear but it may prove a very interesting reagent to use in probing IL-1 signal transduction.

These toxin findings therefore suggest that the nature of the IL-1 activated G protein and its importance in IL-1 action await clarification.

IL-1, second messengers and protein kinases

Most cell activators lead to a change in the levels of specific second messengers, such as cAMP or diacylglycerol, within minutes of binding receptor. This ultimately leads to a change in the activity of a specific protein kinase which phosphorylates discrete substrates ultimately leading to the cellular response. cAMP and diacylglycerol are activators of protein kinase A and C, respectively. There is disagreement as to whether IL-1 can change second messengers in cells. In agreement with some laboratories I have been unable to detect increases in either cAMP or diacylglycerol [4]. However, other investigators have detected changes in cAMP in lymphocytes and fibroblasts [12, 13]. There have also been reports on IL-1 increasing diacylglycerol levels in cells, although the phospholipid source varies. In T cells it is phosphatidylcholine, in macrophages, phosphatidylinositol and in mesangial cells, phosphatildylethanolamine.

The significance of these changes to the final cellular response to IL-1 is ill-defined. Reports have appeared suggesting both protein kinase A and protein kinase C involvement in IL-1 action [12, 13]. Protein kinase A is unlikely to be important for IL-1 signalling however as other agents which activate this kinase do not mimic IL-1. Protein kinase C activation by IL-1 was more likely since phorbol myristae acetate (PMA), which activates protein kinase C, can induce many responses similar to IL-1. However, studies on two proteins which are phosphorylated in response to IL-1 in fibroblasts, the small heat shock protein hsp27 and the EGF receptor, strongly point to the lack of involvement of protein kinase C in these phosphorylations [14, 15]. It is likely that IL-1 is activating a novel protein kinase, distinct from protein kinase A or C in fibroblasts [15].

IL-1 induces transcription factors

Although IL-1 has been shown to increase the phosphorylation of proteins such as hsp27 and the EGF receptor, proteins directly involved in transcription are likely to be more relevant targets for phosphorylation, since the final cellular response to

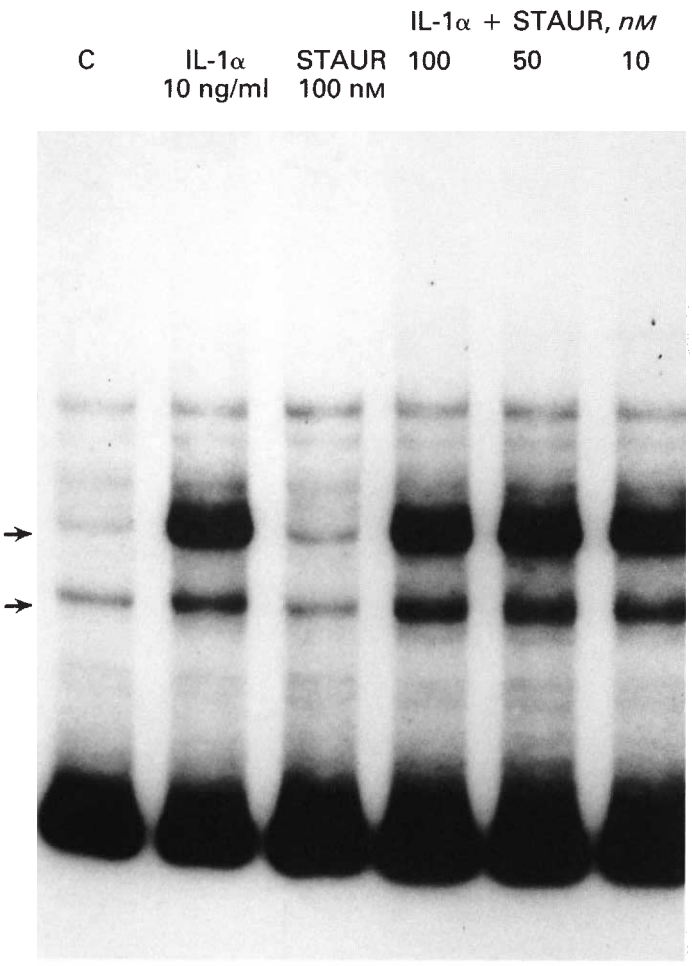

Fig. 3. *Effect of staurosporine on IL-1-induced NFκB in EL4 cells.* EL4 cells were first preincubated with the indicated concentrations of staurosporine, washed and then assessed for NFκB induction as described in the legend to Figure 2.

IL-1 is a change in gene expression. Changes in transcription are mediated by DNA binding proteins termed transcription factors, and several are known to be regulated by phosphorylation. Two are of particular interest with regard to IL-1: NFκB and AP1. Activation of both of these involves phosphorylation [16, 17], and both are induced by IL-1 [18, 19]. AP1 and NFκB sites have been detected in several IL-1 responsive genes. Genes containing AP1 sites include IL-2 and collagenase genes whilst those containing NFκB include IL-2 receptor and serum amyloid A. The IL-2 gene contains sites for both factors [reviewed in 20].

I have been studying the induction of NFκB by IL-1 in EL4 cells. IL-1 is a very strong inducer of this transcription factor in these cells. Figure 2 shows how an increase in NFκB is detectable from five minutes, peaks at 15 minutes and then decreases. This transient response is very similar to the changes in phosphorylation of the EGF receptor and suggests that phosphorylation is involved. The increase was not mimicked by forskolin, a protein kinase A activator, and while PMA induced a change, the time course and pattern of induction was very different (not shown). Also, there was still a response to IL-1 in protein kinase C depleted cells, and staurosporine, a potent inhibitor of protein kinase C, did not inhibit IL-1 (Fig. 3). These results strongly suggest that neither protein kinase A nor C are important in IL-1 action here. It is possible that a novel kinase, similar to that described in fibroblasts is involved.

The mechanism of induction of NFκB by IL-1 is currently under investigation. A general model of activation by PMA has been presented by Ghosh and Baltimore [16]. This involves phosphorylation of an inhibitory protein bound to the NFκB complex, IκB, which then dissociates, allowing the transcription factor to translocate to the nucleus and bind to its cognate sequence. Whether this occurs in response to IL-1 awaits determination.

Conclusions

Clearly the steps leading from IL-1 binding to cells to a change in gene expression are highly complex. Changes in G protein, protein kinase and transcription factor activity are likely to be involved. Determining the molecular details of these events presents a major challenge and remains an essential target for our understanding of this important molecule.

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References

1. Arai K, Lee F, Miyajima, A. Miyatake S, Arai N, Yokota T: Cytokines: Coordinators of immune and inflammatory responses. *Ann Rev Biochem* 59:783–836, 1990
2. Martin M, Resch K: Interleukin 1: More than a mediator between leukocytes. *Trends Pharmacol Sci* 9:171–177, 1988
3. Di Giovane FS, Duff GW: Interleukin 1, the first interleukin. *Immunol Today* 11:13–18, 1990
4. O'Neill LAJ, Bird TA, Saklatvala J: Interleukin 1 signal transduction. *Immunol Today* 11:392–394, 1990
5. Dower SK, Urdal DL: The interleukin 1 receptor. *Immunol Today* 8:46–51, 1987
6. Bomsztyk K, Sims JE, Stanton TH, Slack J, McMahan CJ, Valentine MA, Dower SK: Evidence for different IL-1 receptors in murine B- and T-cell lines. *Proc Natl Acad Sci USA* 86:8034–8038, 1989
7. Gilman AG: G proteins; transducers of receptor generated signals. *Ann Rev Biochem* 56:615–649, 1987
8. O'Neill LAJ, Bird TA, Gearing AJH, Saklatvala J: Interleukin 1 signal transduction. Increased GTP binding and hydrolysis in membranes of a murine thymoma cell line (EL4). *J Biol Chem* 265:3146–3152, 1990
9. Chedid M, Shirakawa F, Naylor P, Mizel SB: Signal transduction pathway for IL1. Involvement of a pertussis toxin sensitive GTP binding protein in the activation of adenylyl cyclase. *J Immunol* 142:4301–4306, 1989
10. Dobson PRM, Plested CP, Jones DR, Barks T, Brown BL: Interleukin 1 induces a pertussis toxin-sensitive increase in diacylglycerol accumulation in mouse thymoma cells. *J Mol Endocrinol* 2:R5–R8, 1989
11. Milligan G: Techniques used in the identification and analysis of function of pertussis toxin-sensitive guanine nucleotide binding proteins. *Biochem J* 255:1–13, 1988
12. Shirakawa F, Yamashita U, Chedid M, Mizel SB: cAMP an intracellular second messenger for interleukin 1. *Proc Natl Acad Sci USA* 85:8201–8207, 1988
13. Munoz E, Buetner U, Zubiaga A, Huber BT: IL1 activates two separate signal transduction pathways in helper type II cells. *J Immunol* 144:964–969, 1990

14. KAUR P, WELCH W, SAKLATVALA J: IL1 increases the phosphorylation of the small heat shock protein hsp27. *FEBS Lett* 258:269–273, 1989
15. BIRD TA, SAKLATVALA J: Down-modulation of EGF receptor affinity in fibroblasts treated with I11 or TNF is associated with phosphorylation at a site other than Thr 654. *J Biol Chem* 265:235–240, 1990
16. GHOSH S, BALTIMORE D: Activation in vitro of NFκB by phosphorylation of its inhibitor, IκB. *Nature* 344:678–682, 1990
17. BOYLE WJ, SMEAL T, DEFIZE LHK, ANGEL P, WOODGETT JR, KARIN M, HUNTER T: Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA binding activity. *Cell* 64:573–584, 1991
18. OSBORN L, KUNKEL S, NABEL G: TNF and IL1 stimulate the human immunodeficiency virus enhancer by activation of NFκB. *Proc Natl Acad Sci USA* 86:2336–2342, 1989
19. MUEGGE J, WILLIAMS TM, KANT J, KARIN M, CHIU R, SCHMIDT A, SIEBENLIST U, YOUNG H, DURUM S: IL1 co-stimulatory activity on the IL2 promoter via AP1. *Science* 246:249–251, 1989
20. MUEGGE K, DURUM SK: Cytokines and transcription factors. *Cytokine* 2:1–8, 1990